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Philip G. Thorne and Daniel C. Leggett

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PREFACE

This report was prepared by Philip G. Thorne, Research Physical Scientist, Geological Sciences Division, and Daniel C. Leggett, Research Chemist, Geochemical Sciences Division, U.S. Army Cold Regions Research and Engineering Laboratory (CRREL), Hanover, New Hampshire.

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Investigations of Explosives and Their Conjugated Transformation Products in Biotreatment Matrices

PHILIP G. THORNE AND DANIEL C. LEGGETT

INTRODUCTION

Previous work on biotreatment of TNT-contaminated soils indicates that TNT is rapidly converted to solvent-extractable transformation products and to residues that are conjugated in a non-solvent-extractable form (Kaplan and Kaplan 1982, Caton et al. 1994, Pennington et al. 1995). Plants grown hydroponically in TNT solutions metabolized TNT reductively and transformation residues were partially conjugated in a non-solvent-extractable form (Palazzo and Leggett 1986a, b; Harvey et al. 1990). A considerable quantity of the two aminodinitrotoluene (ADNT) transformation products was released by acid hydrolysis of the harvested plants after first extracting unconjugated transformation products with benzene (Palazzo and Leggett 1986a, b).

In experiments at Oak Ridge National Laboratory, base hydrolysis following solvent extraction recovered an additional 60% of the applied radiolabel from TNT/soil composts after 90 days (Caton et al. 1994). Although the hydrolysates were not analyzed for specific TNT transformation products, the analogy between living plants and remediation systems containing mostly plant matter and wastes suggested that standard high-pressure liquid chromatography (HPLC) analysis of hydrolysates could be used to identify and quantify conjugated TNT residues in compost and digester sludge.

TNT transformation and disposition in the environment has been thoroughly discussed in several reviews (McCormick et al. 1976, Walsh 1990, Gorontzy et al. 1994). The biological reduction of the nitro groups on TNT proceeds through nitroso and hydroxylamine intermediates to two

aminodinitrotoluene isomers (ADNTs), 4-amino-2,6-dinitrotoluene (4ADNT) and 2-amino-4,6-dinitrotoluene (2ADNT), and two diamino-nitrotoluene isomers (DANTs), 2,4-diamino-6-nitrotoluene (2,4DANT) and 2,6-diamino-4-nitrotoluene (2,6DANT). The condensation of hydroxylamine intermediates to form azoxy dimers has also been reported (McCormick et al. 1976, Kaplan and Kaplan 1982, Spanggord et al. 1995). The reduction pathways for TNT appear to be essentially the same in a wide diversity of species and environmental systems, including mammals, higher and lower plants, bacteria, soil, activated sludge, and compost. However, the predominant amino isomer is 4ADNT in aerobic systems and 2ADNT in anaerobic systems (Carpenter et al. 1978, Pennington 1988, Harvey et al. 1990).

The experiments of Thorn et al. (1996 a, b) using ^{15}N -NMR to study covalent conjugation of the aromatic amine aniline to humic substances and whole peat provide the best model for explaining the fate of aromatic amine and diamine TNT transformation products. Aniline was reacted with purified humic and fulvic acids, silt loam soil, and peat. Analyses of the solid- and liquid-state spectra proved that aniline was readily incorporated into soluble and insoluble humic materials through the formation of anilinoquinone, anilinoquinone, anilide, heterocyclic, and imine bonds by nucleophilic addition reactions. Preliminary experiments with aniline/humic conjugates indicate that the imine and anilide bonds are hydrolyzable, while the anilinoquinone, anilinoquinone, and heterocyclic N-bonds are not. Recently, Thorn (1997) showed that the same types of bonds are formed when the ADNTs and DANTs are reacted with soil humic acids. Because both

compost and digester systems favor the breakdown and humification of plant material, it seems likely that a mixture of reactive carbonyl moieties with metabolically reduced TNT should produce large, heterogeneous polymers that are resistant to further degradation.

The transformation of RDX in biotreatment systems is thought to proceed through reduction of nitro groups to yield nitroso-RDX compounds. This is followed by ring cleavage and the formation of methanol, formaldehyde, hydrazines, and CO₂ (McCormick et al. 1981). Degradation of RDX and HMX in microbial cultures has also been studied (Binks et al. 1995, Roberts et al. 1996). The formation of hydroxylated metabolites from cleaved rings suggests that there are pathways that could lead to conjugated transformation products. This is in contrast to living plant systems, where RDX is accumulated in tissues without transformation or apparent conjugation (Harvey et al. 1991, Checkai and Simini 1996).

The objectives of this work were to evaluate how various analysis methods differentiate between free and conjugated explosives and their transformation products in composted soil and digester sludge and to investigate the time course of transformation and conjugation.

MATERIALS AND METHODS

Composts and digester sludges

Compost samples from aerated windrow composting pilot studies and full-scale treatments at Umatilla Army Depot Activity, Hermiston, Oregon, were obtained from Black and Veatch Waste Sciences, Tacoma, Washington. The compost-amendment mixture was composed of (% by volume) 30% cow manure, 25.4% sawdust, 25.4% alfalfa, 14.3% chopped potato waste, and 4.9% chicken manure. The final compost mixture consisted of 70% amendments and 30% explosives-contaminated sediment excavated from a dried-out explosives waste lagoon. Samples of composted amendments that contained 10% uncontaminated sediments were also available as controls. Digester sludge was from pilot-scale studies performed by Remediation Technologies (Seattle, Washington) at U.S. Naval Submarine Base, Bangor, Washington. Anaerobic, slurry digestion was performed in lined pits. Contaminated soil, water, an organic carbon source (potato starch), and an inoculum of microbes were mixed in proportions to create a slurry that allowed con-

tinuous mixing and heating to above 20°C. Oxygen initially present in the system was soon consumed and reducing conditions of less than -100 mV were maintained for 90 days (Tuomi 1995).

Analytical methods

Solvents used for extractions and analysis were HPLC grade from Alltech Associates, Inc. (Deerfield, Illinois). Concentrated H₂SO₄, Na₂HPO₄, and NaOH were reagent grade from J.T. Baker, Inc. (Phillipsburg, New Jersey). Solid-phase extraction cartridges (Sep-Pak Poropak_{RDX}) were from Waters Corp. (Millford, Massachusetts).

HPLC analysis was performed using either a Spectra-Physics system (8875 autosampler, 8800 pump, 8490 detector, Hewlett-Packard 3396 integrator) (San Jose, California) or a Waters system (717 autosampler, 616 pump, 600S controller, 996 photodiode array detector, Millennium workstation) (Medford, Massachusetts). A Phenomenex (Torrance, California) Ultracarb 5 ODS(20) (4.6-mm × 250-mm, 5-μm) reverse-phase column with an Alltech Associates, Inc. (Deerfield, Illinois) C-18 guard cartridge was used for the analytical separations. The aqueous/methanol (volume %/volume %) gradient elution time steps were as follows: start at 85/15, ramp to 65/35 at 8 minutes, ramp to 42/58 at 10 minutes and hold for 13 minutes, ramp to 0/100 at 28 minutes and hold for 7 minutes, ramp down to 85/15 at 40 minutes and hold for 10 minutes before the next injection. The flow rate was 0.8 mL/minute. Quantification was by peak height performed at 254 nm, while peak identities and purities were assessed by comparing sample and standard UV spectra (200–400 nm) from a diode array detector, and retention times. Further confirmation of analyte identities was performed using a Supelco (Bellefonte, Pennsylvania) cyanopropyl (CN) (4.6-mm × 250-mm, 5-μm) column that separates the analytes in a different order than the ODS column. The detection limits for the analytes were approximately 0.1 mg/kg of dry compost.

Standards for HPLC analysis were made from Standard Analytical Reference Materials obtained from the U.S. Army Environmental Center (Aberdeen, Maryland), except for 2,4DANT and 2,6DANT, which were supplied by Dr. Ronald Spanggord, SRI International (Menlo Park, California).

Solvent extraction

Compost samples were air-dried overnight, then ground to pass a 2-mm screen. Digester slud-

ges contained about 50% solids by weight and had become aerobic by the time of analysis. Sludge samples were air-dried overnight prior to analysis. Subsamples weighing 2.00 g were added to 40-mL glass vials, which were sealed with Teflon-lined caps. A 10-mL aliquot of HPLC-grade acetonitrile was added and the sample mixed on a vortex mixer for one minute. The vials were placed in an ultrasonic bath and sonicated overnight at 30°C. After sonication, a 10-mL aliquot of aqueous calcium chloride solution (5 g/L) was added to hasten flocculation, the sample vortexed briefly, and centrifuged at 2000 g for five minutes. The aqueous CaCl_2 /acetonitrile extract was decanted, filtered, and saved for HPLC analysis. The vials were refilled with fresh CaCl_2 /acetonitrile solution, vortexed, centrifuged, and decanted repeatedly (usually three times, was adequate) until no more unbound explosives residues or free amino transformation products were detectable by HPLC. The resulting solvent-extracted residues were decanted and air-dried overnight before hydrolysis experiments were initiated.

Acid hydrolysis

The first of two hydrolysis procedures was an acid hydrolysis. An air-dried residue from the acetonitrile extraction was transferred to a 22-mL vial, 10 mL of 50% aqueous H_2SO_4 was added, the sample vortexed for one minute, then placed into an ultrasonic bath for six hours at 30°C. Following sonication, the vial was centrifuged at 2000 g for five minutes and a 5-mL aliquot of the acid digest removed and neutralized by adding 100 mL of aqueous, 1.2 M Na_2HPO_4 (pH 8.4). The resulting solution (pH 6.5) was pulled through a 6-mL Sep-Pak Porapak_{RDX} cartridge at 10 mL/minute using vacuum. The cartridge was washed with an additional 20 mL of reagent-grade water to remove salts, then evacuated for five minutes to remove residual water. A 5-mL aliquot of acetonitrile was added and allowed to drip through the cartridge at 5 mL/minute. Reagent-grade water (5 mL) washed through the cartridge into the acetonitrile extract. The mixture was diluted to 10.0 mL using reagent-grade water and analyzed by HPLC.

Base/acid hydrolysis

The second hydrolysis procedure combined a base and acid hydrolysis. Solvent-extracted and air-dried samples from the Day-15 compost and a time series of the digester sludges were transferred to 22-mL vials and treated with 10 mL of 0.5 M NaOH. The samples were vortex-mixed for three

minutes and sonicated overnight at 30°C. The vials were centrifuged and a 5-mL aliquot of each basic extract of the Day-15 compost was removed and diluted to 10 mL with concentrated H_2SO_4 . The remaining 5 mL of basic extract was left in the vial with the residue and 5 mL of concentrated H_2SO_4 was added. Acidified residues were sonicated for six hours at 30°C, centrifuged, and 5-mL aliquots removed and neutralized with 1.2 M Na_2HPO_4 . As a precaution, 3,5-dinitroaniline (3,5-DNA) was added as a surrogate to the neutralized digests to monitor recovery from the Sep-Pak cartridges.

Acid hydrolysis of base-hydrolyzed residues was modified to eliminate the spattering of digestion mixture when concentrated acid was added too quickly. A sample of Day-10 compost was solvent-extracted, rinsed, and base-hydrolyzed as above. A 5-mL aliquot of -15°C 50% H_2SO_4 was added to the 5 mL of base-hydrolysis mixture. The heat generated using this procedure did not cause spattering.

Recoveries

Spike-recovery experiments were performed by adding seven explosives and transformation products dissolved in acetonitrile to field-moist composts, digester sludge, and sand. The spiked concentrations ranged from one to three mg/kg (dry material) for one set of triplicate samples, and 25 to 75 mg/kg for an additional unreplicated set. The acetonitrile was evaporated in a few minutes as the mixtures were homogenized. The air-dried samples were extracted with acetonitrile in accordance with the procedure described earlier.

Neutralization of acid digests of residues sometimes produced a white, gelatinous precipitate. A spike-recovery experiment was performed to determine the relationship between final pH and recovery. Residues from solvent-extracted compost samples were spiked at 16 mg/kg with the ADNTs and the DANNTs. The samples were immediately base/acid hydrolyzed. The hydrolysates were subdivided and neutralized to pH 4.0, 5.0, or 6.0. The 3,5-DNA surrogate was added and the neutralized hydrolysate passed through the solid-phase extraction cartridges.

To evaluate the recovery of the base/acid hydrolysis procedure, 0.5 M NaOH was added to two solvent-extracted compost samples and sand. Either the initial explosives (HMX, RDX, TNT) or the TNT transformation products were spiked into the mixtures at concentrations ranging from 25 to 75 mg/kg. The mixtures were sonicated overnight

and then brought to 25% acid by adding -15°C 50% H_2SO_4 followed by sonicating again for six hours. Hydrolysates were neutralized to pH 5.0 with Na_2HPO_4 . The 3,5-DNA surrogate was added immediately before solid-phase extraction. An aqueous base solution with no added solids was also treated with the same procedure.

Analysis for triaminotoluene in anaerobic cultures

One of the transformation products of TNT is triaminotoluene (TAT). It is known to exist in anaerobic systems (Preuss et al. 1993, Ahmad and Roberts 1995); however, its instability and high degree of polarity have made analysis very challenging. A novel separation using a CN column with either an isocratic or gradient elution with a buffered eluent was developed and tested on bench-scale anaerobic cultures.

The isocratic separation was run on a CN column (Supelco 4.6 mm \times 250 mm) at 1.5 mL/minute of 10 mM phosphate (pH 3.2)/acetonitrile (99 volume %/1 volume %). The retention time of the TAT was 7.4 minutes, well away from more polar interferences that occur in digester solutions. TAT is extremely unstable and begins to degrade into multiple compounds that disappear within an hour or two. Standards and samples have to be prepared immediately before analysis. Getting solid TAT completely into solution requires an acetonitrile/buffer mixture. The aqueous samples from digesters must be mixed 50/50 with acetonitrile before injection. A gradient elution that allows the separation of TAT, both diaminonTs, and the two aminonTs as a single merged peak was developed (Table 1).

Table 1. Gradient elution, LC-CN conditions for triaminotoluene. Flow rate 1.5 mL/min.

Time	Milli-Q	ACN	Buffer
0	0	1	99
2	99	1	0
8.5	60	40	0
15	0	1	99
Restart 20.0			
Retention time (minutes)			
Triaminotoluene	4.4		
2,6DANT	9		
2,4DANT	10.8		
2ADNT	12.9		
4ADNT	13.1		

RESULTS AND DISCUSSION

Solvent extraction

The exhaustive, acetonitrile extractions of the time series showed that the concentration of solvent-extractable (free) TNT was rapidly reduced in compost treatments from 2370 mg/kg on Day 1 to 28.6 mg/kg on Day 10, and to <5 mg/kg by Day 20. These results agree with previous analyses of the composts (Caton et al. 1994, Pennington et al. 1995). ADNTs decreased with time, while the DANTs increased for ten days before decreasing (Table 2, Fig. 1). Traces levels of the azoxy transformation products of TNT and the nitroso transformation products of RDX were found in a few of the samples. No hydroxylaminodinitrotoluenes were detected.

When CaCl_2 /acetonitrile was decanted from samples being extracted, about 10% of the solvent was retained by the residues. Each repeat decantation with fresh solvent removed about 90% of the previously retained analytes, but there was no evidence of further dissolution from the solids. Thus, repeat decantations serve only to purify the residue, removing TNT, RDX, and "free" ADNTs and DANTs. This finding agrees with earlier work from Myers.*

The precision of acetonitrile extraction, based on percent relative standard deviation (% rsd) estimates for triplicate determinations showed no clear dependence on either analyte or concentration. Values ranged from <1% to 73%. The high value was an isolated case because the two next highest values were 37% and 25%. Excluding the one high value and the several cases where <1% was found, the remaining 42 estimates gave a pooled rsd of 11.9% with 84 degrees of freedom.

Acid hydrolysis

On the first day after initiation of composting, there were already conjugated ADNTs and DANTs that were released by the acid hydrolysis (Fig. 1). The quantities of conjugated transformation products increased for ten days, then gradually decreased to levels that were approximately equal to, or slightly higher than, the Day-1 level. Samples of this compost that had been stored in large plastic bags at ambient temperature for several months were also analyzed. After this aging, the quanti-

*Personal communication, K.F. Myers, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi, 1995.

Table 2. Umatilla windrow composts, time series, aerated. Overnight sonic acetonitrile extracts (ACN) and six-hour, sonic H₂SO₄ hydrolyses (acid).

	Concentration (mg/kg)										
	TNT	4ADNT	2ADNT	2,6DANT	2,4DANT	TNB	2,4DNT	HMX	RDX	mononitroso-RDX	trinitroso-RDX
CWR7-aerated											
Day 1 ACN extract	2370	291	170	nd*	nd	8.4	3.3	219	1030	nd	nd
% rsd**	<1	8	8			7	4	14	10		
Day 1 ACID hydrolysate	nd	23.6	4.6	nd	15.2	nd	nd	nd	4.4	nd	nd
% rsd		9	9		25				25		
Day 5 ACN extract	1050	210	220	nd	nd	21.2	2	211	982	nd	nd
% rsd	10	73	12			10	16	8	6		
Day 5 ACID hydrolysate	nd	31.2	4.7	1.6	34.4	nd	nd	nd	3.1	nd	nd
% rsd		14	8	4	10				18		
Day 10 ACN extract	28.6	149	20.3	5.3	53.4	nd	nd	217	546	nd	nd
% rsd	5	6	5	16	23			5	4		
Day 10 ACID hydrolysate	nd	63.1	5.1	4.5	75.1	nd	nd	nd	nd	nd	nd
% rsd		4	8	2	9						
Day 15 ACN extract	14.7	24.5	2.2	nd	8.3	nd	nd	123	73.9	3.9	2.1
% rsd	2	3	4		15			2	2	7	19
Day 15 ACID hydrolysate	nd	46.4	3.7	4.2	66.1	nd	nd	nd	nd	nd	nd
% rsd		2	<1	19	5						
Day 20 ACN extract	1.6	9.2	1.3	nd	4.6	nd	nd	80.1	19.3	2.8	3.5
% rsd	25	5	4		37			3	15	9	2
Day 20 ACID hydrolysate	nd	21.2	2	2.4	53.1	nd	nd	nd	nd	nd	nd
% rsd		3	<1	<1	1						
Day 40 ACN extract	3.3	13.3	2.3	nd	nd	nd	nd	67.8	16.4	5.8	4.5
% rsd	14	12	10					13	11	11	<1
Day 40 ACID hydrolysate	nd	31.4	3.5	2.6	34.4	nd	nd	nd	nd	nd	nd
% rsd		7	1	20	4						

*nd = <0.1 mg/kg

** % rsd = percent relative standard deviation of triplicates

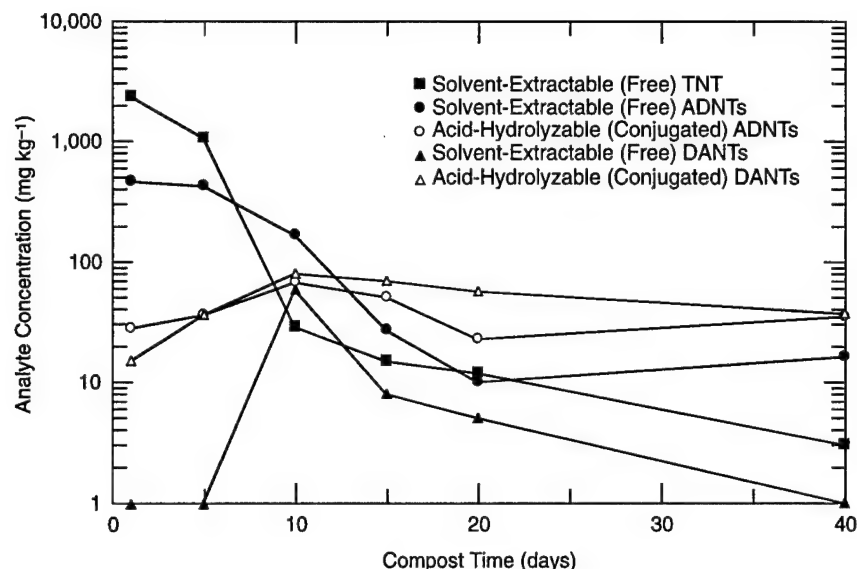


Figure 1. Concentration of free (solvent-extractable) TNT and free and conjugated (acid-hydrolyzable) transformation products recovered from aerated compost.

ties of aminodinitrotoluenes and diaminonitrotoluenes that were released by hydrolysis were further reduced from the Day-40 compost (from 37.8 to 8.1 mg/kg and from 38.0 to 5.5 mg/kg, respectively), demonstrating that further conjugation of these bound residues continues to occur over time.

Acid hydrolysis of solvent-extracted residues degrades the matrix substantially, but results indicate that no additional TNT, RDX, or HMX is released. The stability of TNT, RDX, HMX, and the ADNTs under conditions used for acid hydrolysis has been demonstrated using compost residues. These results confirm that solvent extraction removes all free TNT, RDX, and HMX from sorption sites deep within the matrix as previously shown for soils (Jenkins and Grant 1987), and confirm the results for compost obtained by Myers.*

Base/acid hydrolysis

Base/acid hydrolysis experiments were performed using Day-15 aerated compost and a time series of digester sludge. The quantity of DANTs released from the Day-15 compost by the base hydrolysis was roughly equal to the amount released by the acid-only hydrolysis (57.2 mg/kg

compared to 70.3 mg/kg). The base hydrolysis released fewer of the ADNTs than were released by the acid hydrolysis (19.8 mg/kg compared to 50.1 mg/kg). However, the subsequent acid hydrolysis of the base-hydrolyzed residues released considerably more of the conjugated transformation products (88.8 mg/kg of ADNTs and 284 mg/kg of DANTs) (Fig. 2). The combination of base/acid hydrolysis increased the total recovery of conjugated ADNTs and DANTs by about a factor of three over acid hydrolysis alone.

Results from acetonitrile extraction of the digester time-series sludges shown in Figure 3 agree with previous determinations (Tuomi 1995). Analysis of the extracts and hydrolysates revealed a pattern of explosives degradation and transformation product conjugation that was similar to that found in the composts.

Control composts (composts with the same starting materials but free of explosives residues) were also extracted, hydrolyzed, and analyzed. Retention times and spectra of all peaks, including the unretained solvent peak, were compared to those from sample composts. When this was done, we did not detect any unidentified TNT transformation products released by hydrolysis.

Recoveries

The acetonitrile extractions of sand produced recoveries of RDX, HMX, TNT, and 2ADNT from 92% to 97% (Table 3). Recovery of 4ADNT was

*Personal communication, K.F. Myers, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi, 1995.

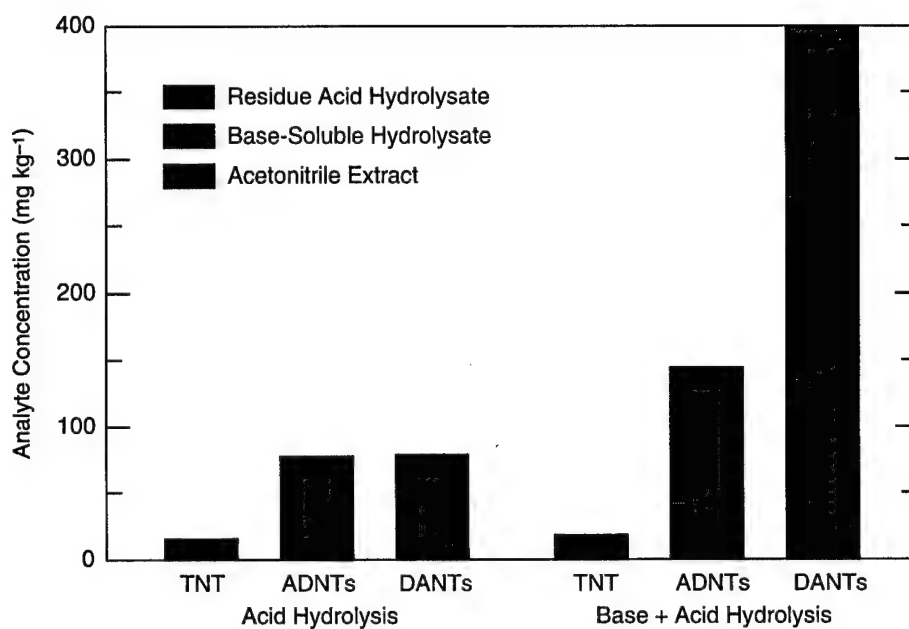


Figure 2. Comparison between the recoveries of ADNT and DANT transformation products from Day-15 aerated compost using acetonitrile extraction, acid digestion, and base/acid digestion.

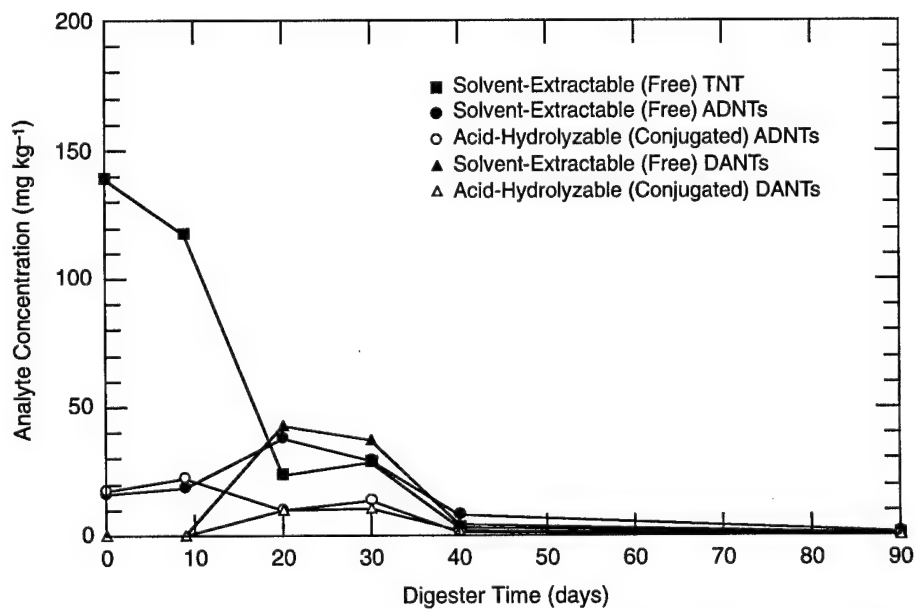


Figure 3. Concentration of free (solvent-extractable) TNT and free and conjugated (base/acid-hydrolyzable) transformation products recovered from digester sludge.

Table 3. Percent recoveries of spikes by acetonitrile extraction.

<i>1-3 ppm spikes</i>								
	TNT	4ADNT	2ADNT	2,6DANT	2,4DANT	TNB	HMX	RDX
Compost								
% recovery	35	91	88	45	27	58	94	97
% rsd-triplicates	16	8	11	9	12	58	7	11
Digester-Day 90								
% recovery	88	25	40	21	7	96	103	90
% rsd	10	8	40	13	12	0	7	2
Sand								
% recovery	93	82	97	27	11	86	92	97
% rsd	3	1	3	8	9	2	2	2
25-75 ppm spikes								
Compost								
% recovery	72	58	82	92	69	64	82	103

82%. The DANTs were recovered at less than 27%. The recoveries from compost were greater than 94% for the nitramines (RDX and HMX); however, the recovery of TNT dropped to 35%. This suggests that TNT is transformed and conjugated rapidly by compost. The recoveries of the transformation products were similar to the recoveries from sand, but these values could be inflated by material initially added as TNT. Recoveries from

digester sludge were 90% and above for the nitramines and 88% for TNT. The recoveries of the TNT transformation products were less than 40% for the aminodinitrotoluenes and less than 21% for the diaminonitrotoluenes. Reproducibility for these recovery estimates was best in sand (rsd = 5.0%) and about 10% for compost and digester sludge. When the compost was spiked at concentrations ranging from 25 to 75 mg/kg, recoveries

Table 4. Effect of neutralization pH on percent recovery of hydrolyzed spikes.

<i>% recovery of spikes</i>					
	4ADNT	2ADNT	2,6DANT	2,4DANT	3,5DNA
Compost A					
pH 4	nd*	nd	14	42	74
pH 5	nd	nd	22	76	58
pH 6	nd	nd	19	76	10
Compost B					
pH 4	nd	nd	10	29	75
pH 5	nd	nd	12	39	51
pH 6	nd	nd	8	29	28
Compost C					
pH 4	nd	nd	20	70	85
pH 5	nd	nd	18	72	75
pH 6	nd	nd	12	39	57
Compost D					
pH 4	nd	nd	16	63	92
pH 5	nd	nd	19	76	104
pH 6	nd	nd	9	40	61

*nd= <0.4%

Table 5. Recovery of transformation products from compost using 100% or 50% H₂SO₄ after base hydrolysis.

	Concentration (mg/kg)			
	4ADNT	2ADNT	2,6DANT	2,4DANT
100% acid	63.1	5.1	4.5	75.1
50% acid	19.8	<0.1	3.1	97.1

of the TNT and diaminonitrotoluenes rose to greater than 69%, while the recoveries of the aminodinitrotoluenes were less than 82%. The difficulty in recovering ADNTs, even in acidified water matrices, has been reported previously (Jenkins et al. 1995).

It was apparent (Table 4) that the pH must be carefully controlled. A pH of 5.0 is recommended to provide a compromise between absolute recovery of all analytes and a reasonable recovery of the 3,5-DNA surrogate so that solid-phase extraction recoveries can be estimated independently of the hydrolysis/neutralization losses.

Acid hydrolysis from addition of cold 50% acid resulted in lower recoveries of TNT transformation products than were recovered when 100% sulfuric acid was used (Table 5). Nevertheless, we recommend the use of 50% acid because of the potential for injury caused by spattering of hot acid when 100% acid is used. It is important when assessing these analytical recoveries to realize the

dynamic nature of this system. The ADNT and DANT transformation products of TNT are formed, conjugate, and further bind as biotreatment of these soils takes place. Target analytes spiked into these matrices are unstable and recoveries are hence problematic.

None of the untransformed explosives TNT, RDX, and HMX were detected in the hydrolysis spike recovery tests because of their instability in basic solution. Results (Table 6) showed that the procedure recovered from 73% to 90% of the ADNTs and from 88% to 93% of the DANTs when no solids were present. However, in the presence of compost, no ADNTs were detected and the recoveries of the DANTs dropped to 18%–62%. In the presence of sand, the recovery of the ADNTs ranged from 5 to 9% while the recovery of the DANTs was from 9% to 40%. For all mixtures that contained solids, the recovery of the 3,5-DNA surrogate that was added to the neutralized hydrolysates immediately before solid-phase extraction ranged from 72 to 88%.

The ability to hydrolyze and recover unbound transformation products from composts changes over the time course of the treatment. Hydrolysis of the aerated composts released the greatest quantities of TNT transformation products during the midpoint of the composting process (Table 2). Hydrolysis of the Day-40 compost released fewer transformation products, presumably because of further conjugation with formation of functional groups that are more resistant to hydrolysis. The incomplete recoveries of TNT and transformation products that were spiked to composts from the

Table 6. Percent recoveries from base/acid hydrolyzed solution (no solid), compost, and sand. No TNT, RDX, or HMX were recovered.

	% recovery of spikes				
	4ADNT	2ADNT	2,6DANT	2,4DANT	3,5DNA
Solution	90	73	88	93	106
% rsd-triplicates	3	11	11	11	8
Compost A	nd*	nd	18	62	72
% rsd			21	22	14
Compost B	nd	nd	39	56	87
% rsd			32	5	4
Sand	9	5	9	40	88
% rsd	<1	<1	<1	6	5

*nd = <0.4%

end of full-scale operations indicate that fully developed compost has a residual capacity to reduce TNT and conjugate the resulting transformation products. This does not explain the loss of transformation products in the presence of sand with negligible organic carbon (<0.2%), but is consistent with losses of these compounds reported for acidified water (Jenkins et al. 1995). None of the azoxy transformation products of TNT were detected.

Analysis for triaminotoluene in anaerobic cultures

Anaerobic cultures from an experiment at the U.S. Army Construction Engineering Research Laboratories were received in anaerobic serum bottles. The TNT amended cultures had been incubated until all of the TNT, aminoDNTs, and diaminoNTs had been depleted. A sample withdrawn by syringe, mixed with acetonitrile, and analyzed immediately contained TAT, but no other reduction products. An unamended control bottle was blank. The vessel was opened and 1.5 g of the solid material was solvent-extracted and base/acid hydrolyzed as above. No TNT or any of its reduction products were recovered by any of the treatments.

CONCLUSIONS

Previous researchers using radiolabels found that the majority of TNT reduction products were bound in non-solvent-extractable forms. Fractionation of composts (Caton et al. 1994, Pennington et al. 1995) and soils (Comfort et al. 1995) into functional categories indicated that 25–60% of the unextractable residues were associated with fulvic and humic acids. Our results agree with these findings while providing the necessary identifications of hydrolyzed analytes. In concurrent research, Thorn (1997) is using NMR spectroscopy to identify the bonds that are formed when ^{15}N -labeled aminoDNTs and diaminoNTs are reacted with humic substances, soils, and peat. Identification of which bonds are hydrolyzable will be accomplished by comparing the NMR spectra of the conjugation products before and after hydrolysis. Preliminary experiments with aniline/humic conjugates indicate that the imine and anilide bonds are hydrolyzable by base or acid, while the anilinoquinone, anilinohydroquinone, and heterocyclic N-bonds are not. Thus, it appears that the increased recovery of conjugated transformation

products by the base/acid hydrolysis may be due to the disruption of the humic polymers and increased accessibility of bonds.

The transformation of TNT to solvent-non-extractable reduction products appears to go through two stages of covalent conjugation in compost and digester sludge. In the first stage, a significant percentage of the bonds are hydrolyzable by acid or base alone or a combination of base and acid. As processing continues, a second stage occurs in which the bonds are either altered to form different functional groups that are non-hydrolyzable, or additional bonds form as the bound transformation products are further reduced. The termination of treatment as soon as solvent-extractable TNT concentrations drop below a specified action level may stop the conjugation processes at the first, hydrolyzable stage. This may result in the potential for long-term releases of TNT metabolites that might not occur if composting or digesting is continued into the second stage of nonhydrolyzable conjugation. The most current study of the multispecies toxicity of the fully developed composts from the Umatilla pilots (Gunderson et al. 1997) shows that long-term ecological effects are minimal. Some nonlethal effects on plants were observed.

Spike-recovery studies indicated that current methods do not adequately quantify TNT transformation products, particularly at the low concentrations that characterize remediation goals. Current methods are sufficient to remove all of the analytes that are extractable. These may comprise the explosives remaining undissolved in the soil, plus the pool of transformation products that are in the aqueous phase or sorbed and not yet conjugated to the solid phase of the organic matter. However, the transformation products that are released by hydrolysis represent a dynamic pool of weakly conjugated amines that are subject to further reduction and/or bond transformations that eventually become unhydrolyzable as humification continues. Therefore, analyses of bioremediation matrices should be considered as a qualitative descriptor of the progress of humification and the capacity to covalently conjugate transformation products rather than as a quantitative measure of the absolute amounts of various analytes present.

The purpose of the base/acid hydrolysis is to break the bonds between TNT transformation products and organic carbon biopolymers such as humic acid. Detection limit studies showed that a minimum of 0.1 mg of transformation product

would have to be released from 1 kg of organic carbon. Therefore, the method is applicable only to high-carbon matrices like compost, digester sludge, or plant and animal tissues. In soil or aquifer materials that contain a few percent or less of organic carbon, there are not enough hydrolyzable products potentially available for analysis by this method.

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